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Effects of an Essential Oil from the Bark of Croton cajucara Benth. on Experimental Gastric Ulcer Models in Rats and Mice

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Abstract

Croton cajucara Benth. (Euphorbiaceae) is widely used in Amazonian folk medicine for the treatment of a wide range of gastrointestinal symptoms. The essential oil from its bark was investigated for acute toxicity in mice and for its ability to prevent the formation of ulceration of the gastric mucosa in different models of experimentally induced gastric ulcer in mice and rats.

When previously administered orally at a dose of $100\,mg\,kg^{-1}$, the essential oil significantly reduced ($P < 0{\cdot}01$) the gastric injury induced by hypothermic restraint stress (48%), indomethacin (47%), ethanol (86%) and pylorus ligature models (87%) in rats. In the HCl/ethanol-induced gastric ulcer model in mice, at oral doses of 100 and $200\,mg\,kg^{-1}$, the essential oil from *C. cajucara* significantly reduced ($P < 0{\cdot}01$) the formation of gastric lesions by 52% and 67%, respectively, when compared with the control group. In rats submitted to pylorus ligature, the essential oil given orally increased the volume of gastric juice when compared with the control group ($P < 0{\cdot}01$). When the essential oil ($100\,mg\,kg^{-1}$) was administered intraduodenally to mice, significant modifications were found in gastric parameters such as pH and total acid content after oil treatment. We observed significant changes ($P < 0{\cdot}01$) in gastric juice parameters such as an increase in volume and a decrease in gastric acidity (pH and total acid content). The acute toxicologic effects of the essential oil from *C. cajucara* were assessed in mice. The LD50 values were $9{\cdot}3\,g\,kg^{-1}$ by the oral route and $680\,mg\,kg^{-1}$ by the intraperitoneal route.

The good yield of essential oil obtained from dried *C. cajucara* bark (1%) as well as its anti-ulcerogenic activity and low toxicity suggest that pharmacological studies of this substance as a potential new anti-ulcerogenic drug are warranted.

An aromatic bitter tea made from the bark and leaves of *Croton cajucara* Benth. (Euphorbiaceae), commonly known as sacaca, is widely used in Amazonian folk medicine for the treatment of a wide range of gastrointestinal symptoms (Di Stasi et al 1989). We recently reported the anti-ulcerogenic activity of *trans*-dehydrocrotonin (DHC), the principal furane diterpene isolated from *C. cajucara* bark, in different ulcerogenic models in mice and rats (Rodriguez et al 1999; Souza Brito et al 1998) and later described the possible anti-ulcerogenic mechanisms involved in the action of DHC (Hiruma-Lima et al 1999). The acute and subchronic toxicity of this compound was also studied by our group in in-vivo and in-vitro assays that showed it to possess relatively low oral toxicity when administered for a short period of time (35 days).

In addition to DHC, the bark also contains 1% of a very pleasant essence composed principally of sesquiterpenes. Thus, in the present pharmacological studies we analysed the anti-ulcerogenic activity of this essential oil in indomethacin-, hypothermic restraint stress-, ethanol- and pylorus

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ligature-induced gastric ulcer in mice and rats. In the HCl/ethanol model in mice, we studied whether the effect of the essential oil was dose-dependent and compared it with that of lanzoprazole and omeprazole. Parameters of gastric acid secretion such as gastric acidity and gastric juice volume were analysed in animals submitted to the Shay method, which were treated with essential oil by the oral and intraduodenal route. Moreover, the in-vivo acute toxicological effect of the essential oil from the bark of *C. cajucara* was also determined.

Material and Methods

Animals

Male Wistar rats (250–300 g) and male albino Swiss mice (25–35 g) from the Central Animal House of the Universidade Estadual de Campinas (CEMIB/UNICAMP) were used. The animals were fed a certified Nuvilab CR-a (Nuvital) diet and had free access to water under standard conditions of illumination (12 h dark–12 h light), humidity ($60 \pm 1{\cdot}0\%$) and temperature ($21{\cdot}5 \pm 1{\cdot}0\%$). Fasting was used before all assays because standard drugs or essential oil were administered orally (by gavage) or intraperitoneally using as vehicle a 12% solution of Tween 80 ($10\,mL\,kg^{-1}$). All the protocols were approved by the Ethics Committee of UNICAMP (registered in the Brazilian National Council of Health – Res. 196/96). All the experiments were conducted in agreement with the recommendations of the Canadian Council on Animal Care (Olfert et al 1993).

Drugs

The following drugs were used: cimetidine, lanzoprazole, Tween 80 and indomethacin. All reagents were of a high grade of purity. The substances and reagents were prepared immediately before use.

Preparation and analysis of the essential oil

The stem bark of *Croton cajucara* Benth. was collected at our experimental plantation in Benfica, near Belém, Pará, Brazil. A voucher specimen (number 247) has been identified by Nelson A. Rosa and deposited in the IAN Herbarium in Belém, Brazil. The air-dried and milled bark (20 kg) was subjected to steam distillation for 6 h, a first fraction of 163 mL (F1) was collected after 3 h and a second fraction of 42 mL (F2) at the end of the process. Preliminary GC-FID and GC-MS analyses performed with an Hewlet Packard system using a HP-5 capillary column showed very similar patterns for both F1 and F2 fractions, which were composed mainly of $C_{15}H_{24}$ sesquiterpenes. α-Copaene (20·9%) and cyperene (29·0%) were the main components of F1, as confirmed by ^{13}C NMR spectra measured in a Varian spectrometer operating at 75·4 MHz and using benzene-d_6 as solvent. Complete analyses of the samples are in progress. The F1 fraction was used for the pharmacological tests. F1 was emulsified in 12% Tween 80 before administration to the animals.

In-vivo toxicity

The acute oral toxicity of essential oil from *C. cajucara* bark was determined in male albino Swiss mice which had been fasted for 12 h. Increasing doses of essential oil were administered orally by gavage to groups of 10 animals for each dose level (1, 2·5, 5 and $7{\cdot}5\,g\,kg^{-1}$). Animals receiving the vehicle (12% Tween 80) served as control. The groups were observed at 0, 30, 60, 120, 180 and 240 min after oil administration and then twice a day for the next 14 days. At the end of this period the number of survivors was recorded and the acute toxicologic effect was determined on the basis of mortality, expressed as LD50. The oral LD50 value was obtained using a software based on the method of Litchfield & Wilcoxon (1949).

Anti-ulcerogenic activity

HCl/ethanol-induced ulcer. The anti-ulcerogenic activity of essential oil in HCl/ethanol-induced gastric ulcer was assessed in mice as described by Mizui & Doteuchi (1983). Mice were divided into groups of 6–12 animals and fasted for 24 h before receiving an oral dose of either the vehicle, 12% Tween 80 ($10\,mL\,kg^{-1}$), lansoprazole ($20\,mg\,kg^{-1}$), omeprazole at the same dose or essential oil (50, 100 or $200\,mg\,kg^{-1}$). After 50 min all groups were orally treated with 0·2 mL of a 0·3 M HCl/60% ethanol solution (HCl/ethanol) for gastric ulcer induction. Animals were killed with ether 1 h after the administration of HCl/ethanol, and the stomachs were excised and inflated by injection of saline (2 mL). The stomachs were fixed in 5% formalin for 30 min and opened along the greater curvature. The extension of the lesions was measured, and the ulcerative index is expressed as the sum of all lesions.

Indomethacin ulcer. A total of 17 rats were randomly divided into 3 groups and fasted for 24 h, with free access to water before the experiment.

Thirty minutes after oral administration of essential oil (100 mg kg^{-1}), cimetidine (100 mg kg^{-1}) or 12 % Tween 80 (10 mL kg^{-1}), 30 mg kg^{-1} of indomethacin was subcutaneously administered to unanaesthetized rats from each group according to the method of Hayden et al (1978). Indomethacin was dissolved in 5% sodium bicarbonate. The animals were killed 4 h later, the stomachs removed and opened and the gastric lesions were determined as described above.

Hypothermic restraint stress ulcer. The anti-ulcerogenic activity of *C. cajucara* essential oil was assessed in the hypothermic restraint stress-induced gastric ulcer model in rats according to the method of Levine (1971), with some modifications. Rats were divided into groups of 7 animals each. After 24 h of starvation, the animals received an oral dose of essential oil (100 mg kg^{-1}), cimetidine (100 mg kg^{-1}) or 12% Tween 80 (10 mL kg^{-1}). One hour after the treatment, gastric ulceration was induced by immobilising the animals inside a closed cylindrical cage maintained at 4°C. After 3 h the animals were killed with ether and the stomachs removed and examined for ulcers as described previously.

Ethanol-induced ulcer. The ethanol-induced ulcer assay was carried out in rats according to the method of Morimoto et al (1991). A total of 17 animals were randomly divided into 3 groups and fasted for 24 h, with free access to water before the experiment. One millilitre of 99·5% ethanol was orally administered to the animals which 1 h previously had been treated orally with essential oil (100 mg kg^{-1}), omeprazole (20 mg kg^{-1}) or 12% Tween 80 (10 mL kg^{-1}). One hour after ethanol administration the animals were killed, the stomachs were removed and opened and the ulcerative index was determined.

Table 1. Effects of omeprazole, lanzoprazole and different doses of essential oil from *Croton cajucara* on HCl/ethanol-induced gastric ulcer in mice.

Treatments (p.o.)	Dose (mg kg^{-1})	n	Ulcerative Index (mm)	Inhibition (%)
Control	–	12	32·5 ± 8·37	–
Omeprazole	20	8	17·5 ± 7·62*	46
Lansoprazole	20	7	9·57 ± 3·10*	71
C. cajucara oil	200	7	10·6 ± 2·82*	67
C. cajucara oil	100	6	15·7 ± 2·88*	52
C. cajucara oil	50	7	30·1 ± 10·2	7

Results are expressed as mean ± s.d. Analysis of variance: $F(5,41) = 17{\cdot}1$ $P < 0{\cdot}05$. Dunnett's test *$P < 0{\cdot}01$.

Shay ulcer. A total of 18 rats were randomly divided into three groups and fasted for 24 h, with free access to water. Thirty min after oral administration of essential oil (100 mg kg^{-1}), cimetidine (100 mg kg^{-1}) as positive control or vehicle (12% solution of Tween 80, 10 mL kg^{-1}), a pylorus ligature was performed as described by Shay et al (1945). The animals were killed 4 h later, the abdomen was opened and another ligature was placed around the oesophagus close to the diaphragm. The stomach was removed, inspected internally, and its contents drained into a graduated centrifuge tube and centrifuged at 2000 rev min^{-1} for 10 min. The supernatant volume and pH were recorded with a digital pH meter. The total acid content of gastric secretion was also determined by titration to pH 7·0 with 0·05 N NaOH using a digital burette. Gastric lesions were evaluated by examining the inner gastric surface with a dissecting binocular microscope and the mucosal lesions were counted and scored as described above.

Determination of gastric secretion. A total of 18 mice were randomly divided into three groups and fasted for 24 h with free access to water. The assay was performed by the method of Shay with some modifications (Shay et al 1945). Immediately after pylorus ligature, *C. cajucara* essential oil (100 mg kg^{-1}), cimetidine (100 mg kg^{-1}) as positive control or vehicle, a 12 % solution of Tween 80 (10 mL kg^{-1}), was administered intraduodenally. The animals were killed by cervical dislocation 3 h later and the same procedures as described for Shay ulcer were followed.

Statistical analysis

Results are expressed as the mean ± s.d. Statistical significance was determined by one-way analysis of variance followed by Dunnett's test, with the level of significance set at $P < 0{\cdot}05$.

Results

The LD50 value obtained by probit analysis for oral administration of *C. cajucara* essential oil was 9·26 g kg^{-1} ($r = 0{\cdot}99 \pm 0{\cdot}24$, $n = 10$, $P > 0{\cdot}05$). These data are not shown.

The effects of *C. cajucara* essential oil on induced gastric ulcer were first investigated in mice and the results are shown in Table 1. Oral administration of HCl/ethanol solution to the control group clearly produced the expected characteristic zonal necrotizing mucosal lesions. Essential oil was given orally at doses of 50, 100 and 200 mg kg^{-1}

while omeprazole and lanzoprazole (positive controls) were administered orally at a dose of 20 mg kg^{-1}. The lesion index for the control group of the HCl/ethanol-induced gastric ulcers was 32·5 ± 8·37 mm. The anti-ulcer drugs lanzoprazole and omeprazole and essential oil (200 and 100 mg kg^{-1}) significantly inhibited ulcer formation by 71, 46, 67 and 52%, respectively. Interestingly, no significant differences were observed between the groups treated with 100 or 200 mg kg^{-1} of essential oil or between the group treated with 50 mg kg^{-1} of essential oil and the negative control.

The effects of essential oil on the four assayed methods of induced gastric lesions are shown in Table 2. Oral administration of *C. cajucara* essential oil at a dose of 100 mg kg^{-1} inhibited the appearance of gastric lesions induced by hypothermic restraint stress, indomethacin, ethanol and pylorus ligature. The best inhibitory effect on the ulcerative index was observed in the model of pylorus ligature (87%) followed by the ethanol model (86%). The same relative potency (47% inhibition) was observed for indomethacin- and hypothermic restraint stress-induced gastric lesions.

In the pylorus ligature method, the administration of the essential oil by different routes produced a significant modification in gastric volume, pH and gastric acid content (Table 3). The pylorus-ligated rats treated with essential oil (100 mg kg^{-1}, p.o.) only showed a significant increase in gastric volume compared with the control group. In contrast, cimetidine at 100 mg kg^{-1} significantly reduced gastric-juice volume and acidity and increased gastric pH. However, the essential oil administered intraduodenally to mice at the same dose was effective in inducing a significant increase in gastric juice and pH, and a decrease in total gastric acid.

Discussion

Since no studies of the anti-ulcer activity of *Croton cajucara* are available, the possible effects of this

Table 2. Effects of essential oil from *Croton cajucara* on the four assayed methods of induced gastric lesions in mice and rats.

Animal	Gastric lesion model	Treatment	N	Ulcerative index (mm)	Inibition (%)
Rats	Indomethacin	Control	6	66·0 ± 14·8	–
		Cimetidine	6	6·0 ± 2·6*	91
		Essential oil	5	34·9 ± 9·3*	47
	Ethanol	Control	5	91·8 ± 12·4	–
		Omeprazole	6	32·2 ± 14·3*	65
		Essential oil	6	12·8 ± 4·4*	86
Mice	Ligature	Control	6	17·3 ± 5·43	–
		Cimetidine	5	2·33 ± 2·07*	86
		Essential oil	5	2·20 ± 2·28*	87
	Stress	Control	7	40·6 ± 11·6	–
		Cimetidine	7	21·1 ± 2·54*	48
		Essential oil	7	21·4 ± 4·61*	47

Cimetidine or essential oil from *C. cajucara* were administered orally at a dose of 100 mg kg^{-1} and omeprazole was given orally at a dose of 20 mg kg^{-1}. Values for ulcerative index are expressed as mean ± s.d. Analysis of variance gave indomethacin: $F_{(2\cdot14)} = 51\cdot2$ $P < 0\cdot05$; ethanol: $F_{(2\cdot14)} = 72\cdot6$ $P < 0\cdot05$; ligature: $F_{(2\cdot14)} = 32\cdot5$ $P < 0\cdot05$; stress: $F_{(2\cdot18)} = 15\cdot9$ $P < 0\cdot05$. Dunnett's test $^*P < 0\cdot01$.

Table 3. Effects of essential oil from *Croton cajucara* on biochemical parameters of the gastric juice obtained from pylorus-ligated rats and mice (n = 6).

Animals	Treatments	Route	pH	Gastric juice volume (mL)	Total gastric acid (mEq mL^{-1})
Rats	Control	p.o.	3·21 ± 1·15	2·50 ± 0·77	1·16 ± 0·61
	Cimetidine	p.o.	6·33 ± 1·03**	1·43 ± 0·39**	0·31 ± 0·12**
	Essential oil	p.o.	2·80 ± 0·84	3·90 ± 0·44**	0·64 ± 0·20
Mice	Control	i.d.	3·66 ± 0·52	0·36 ± 0·03	6·02 ± 1·58
	Cimetidine	i.d.	6·13 ± 0·73**	0·65 ± 0·23*	1·87 ± 0·62**
	Essential oil	i.d.	7·17 ± 0·82**	0·72 ± 0·28**	1·36 ± 0·64**

Cimetidine were administered orally (p.o.) to rats or intraduodenally (i.d.) to mice at a dose of 100 mg $^{-1}$. Data are expressed as mean ± s.d. Analysis of variance for rats: $F_{(2\cdot15)}$ for pH = 20·5 $P < 0\cdot05$; $F_{(2\cdot15)}$ for volume = 24·4 $P < 0\cdot05$; $F_{(2\cdot15)}$ for total acid = 7·29 $P < 0\cdot05$. Analysis of variance for mice: $F_{(2\cdot15)}$ for pH = 39·9 $P < 0\cdot05$; $F_{(2\cdot15)}$ for volume = 5·0 $P < 0\cdot05$; $F_{(2\cdot15)}$ for total acid = 35·6 $P < 0\cdot05$. Dunnett's test $^*P < 0\cdot05$ and $^{**}P < 0\cdot01$.

plant are currently being investigated using various ulcer models to determine the potential anti-ulcerogenic profile of this Brazilian medicinal plant (Souza Brito & Nunes 1997).

Our group has previously demonstrated the anti-ulcerogenic properties of DHC isolated from the bark of *C. cajucara*. In the present study, we analysed another bark component possibly involved in the anti-ulcerogenic activity of this plant, namely the essential oil. Previous chromatograph analyses showed that the essential oil has no traces of DHC in its composition. Thus, the possible anti-ulcerogenic effect of essential oil was attributed to its own composition. The essential oil of *C. cajucara* bark did not present significant acute toxicological effects. The oral LD50 was obtained with a 90-fold higher dose than the active anti-ulcer dose ($100\,mg\,kg^{-1}$).

The present study was carried out using different experimental models of gastric ulcer which operate by distinct mechanisms of ulcerogenesis (Desai & Parmar 1994). The preventive effects of the essential oil of *C. cajucara* bark on gastric ulcer induced by the various necrotizing agents studied were investigated in mice and rats. The ulcerative index was significantly lower in essential oil-treated mice (100 and $200\,mg\,kg^{-1}$) compared with the control for HCl/ethanol-induced lesions. Essential oil at an oral dose of $100\,mg\,kg^{-1}$ presented a 50% protection in this pharmacological model. Interestingly, no significant differences were observed between the groups treated with oil at $100\,mg\,kg^{-1}$ and $200\,mg\,kg^{-1}$ or between the groups treated with essential oil at $50\,mg\,kg^{-1}$ and the control.

Hypothermic restraint stress ulcers have been widely used experimentally for the evaluation of anti-ulcer activity in rats because of data reproducibility (Murakami et al 1985). Disturbances of gastric mucosal microcirculation, alteration of gastric secretion and abnormal motility have been considered to be the pathogenic mechanisms responsible for stress-induced gastric mucosal lesions and gastric mucus depletion (Koo et al 1986). However, the most important factor in the genesis of stress ulcer is the increase in gastric acid secretion and this is often termed the aggressive factor (Goa & Monk 1987).

The essential oil ($100\,mg\,kg^{-1}$) significantly protected the gastric mucosa against hypothermic restraint stress-induced ulcers in mice with an effect comparable to that of cimetidine at the same dose.

Ethanol treatment induces solubilization of mucus constituents in the stomach with a concomitant fall in the transmucosal potential difference, and increases Na^+ and K^+ flux into the lumen, pepsin secretion, the loss of H^+ ions and the histamine content in the lumen. This drug also depresses tissue levels of DNA, RNA and proteins, leading to flow stasis in injured areas (Szabo 1987). Moreover, it is well known that ethanol-induced ulcers are not inhibited by anti-secretory agents such as cimetidine, but are inhibited by agents which enhance mucosal defensive factors such as prostaglandin E_2 (Robert et al 1979). In the present study, *C. cajucara* essential oil significantly protected the gastric mucosa against the injury induced by ethanol. These results further indicate that this essential oil may enhance gastric mucosal defensive factors such as mucus, prostaglandins, or both.

Anti-inflammatory agents such as indomethacin reduce gastric cyclooxygenase activity and decrease endogenous prostaglandin levels (Konturek et al 1984). These agents break the mucosal barrier, provoke an increase in gastric mucosal permeability to H^+ and Na^+ ions and a drop in the transmucosal potential difference, and induce the formation of erosions and ulcers (Droy-Lefaix 1988). There is mounting evidence that an increase of certain endogenous prostaglandins can enhance gastric mucosal resistance against ulcerogenic agents such as anti-inflammatory agents (Wallace & Whittle 1985). In this assay, the essential oil from *C. cajucara* was also able to produce a significant reduction of the gastric mucosal damage induced by indomethacin, indicating once again the probable local increase in prostaglandin synthesis. Subsequently we showed the biochemical results obtained after submitting the animals to pyloric ligature. Rats were pretreated orally with essential oil or cimetidine and mice were pretreated with the same doses by the intraduodenal route. The oral pretreatment with cimetidine provoked changes in the acidity and volume of gastric juice. The essential oil only provoked a marked increase in the volume of gastric juice. The intraduodenal administration of both drugs had similar effects on all of the other parameters analysed. Like cimetidine, the essential oil was also effective in reducing gastric acidity and in increasing the volume of gastric juice.

There is evidence of an involvement of prostanoids in the accumulation of fluid in the gastric lumen; prostaglandin E_2 causes a significant increase of volume flow in the stomach (Droy-Lefaix 1988). Moreover it seems that the essential oil of *C. cajucara* exerts a kind of cytoprotective action mainly through a systemic action because this action is present not only when the oil is administered orally, but also when it is administered intraduodenally. Thus, its protective effect does not depend on contact with the gastric mucosa.

The present results clearly indicate that oral administration of essential oil from *C. cajucara* bark produced a significant anti-ulcer effect that was probably due to an increase in mucosal defensive mechanisms such as prostaglandin production. Moreover, the low toxicity level observed in-vivo supports the acute medicinal use of this species with a wide safety range and without severe risks for users.

In conclusion, the oral administration of *C. cajucara* essential oil displayed a significant anti-ulcer activity with no toxicological effects. New experiments are currently underway to determine the composition of the essential oil as well as the anti-ulcer mechanisms involved.

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